REMARKS

Claims 1-3, 5-10, 12, 14, 15 and 30 are pending in this application. Claims 13 and 29 have been canceled herein without prejudice or disclaimer. Claim 1 has been amended herein and new claim 30 has been added.

Support for the amendment to claim 1 is discussed below. Support for new claim 30 may be found in the specification on page 13, lines 1-4.

Interview Summary

Daniel Geselowitz conducted a personal interview with Examiner Katcheves on August 18, 2003. In the interview the Examiner indicated that the cancellation of claim 13 would overcome the rejection under 35 U.S.C. 112, first paragraph. Applicants indicated that they would make claim amendments and present a Declaration under 37 CFR 1.132.

Claims 1-3, 5-10, 12-15 and 29 stand rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for epidermal target cells, does not reasonably provide enablement for hepatic target cells.

The rejection is overcome by the cancellation of claim 13 without prejudice or disclaimer. Claim 13 was the only claim to explicitly recite hepatic target cells.

Claims 1-3, 5-10, 12-15 and new claim 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boyce et al. (U.S. Patent No. 4,940,666) or Rheinwald et al. for reasons of record and those discussed below.

The rejection of pending claims 1, 3, 5-10, 12, 14 and 15 is overcome by the amendments to the claims. The remarks below are also applicable to new claim 30.

Applicants first note that the Examiner here apparently refers to Rheinwald et al. (1977).

In the rejection under 35 U.S.C. 103(a), the Examiner states (page 5, lines 3-5), that "one of skilled in the art would have been motivated to **leave the 3T3 cells** in the initial growth vessel and inoculate the keratinocytes in that vessel" (emphasis added).

However, the present invention is characterized in that **the treated fibroblasts are separated from the culture vessel** so as to leave an extracellular matrix on a surface of said culture vessel, and then epithelial cells are inoculated and cultured in said vessel, as recited in claim 1, as amended. In the original specification, there are descriptions that killed fibroblasts may be preferably separated from the vessel partially, and more preferably entirely (page 5, lines 24-25 in original specification). Applicants submit that the amendment to recite "separating the treated fibroblasts" is therefore supported by the specification. Dependent claims 2 and 3 therefore further limit claim 1 only in the amount of treated fibroblasts that is separated.

On the other hand, the cited references (Boyce USP 4, 940,666) and Rheinwald (Cell, vol. 265 (1977)) teach culturing a mixture of 3T3 cells and keratinocytes, and then removing 3T3 cells from the culture vessel (for example, column 2, lines 43-47 and lines 52-60 in Boyce; and explanation in Table 1 in Rheinwald). Therefore, the step of separating the treated fibroblasts from the culture vessel before inoculating and culturing epithelial cells in the culture vessel represents a difference between the present invention and the references.

In addition, Boyce discloses that "in the absence of 3T3 cells, the human keratinocytes could not even initiate colony formation" (column 2, lines 39-42). Considering the description of Boyce,

persons skilled in the art would have been motivated to leave the fibroblasts in the culture vessel and inoculate the keratinocytes in the culture vessel, rather than to separate the fibroblasts from the culture vessel before inoculating and culturing epithelial cells in the culture vessel. Rheinwald also discloses that "In cell culture conditions, keratinocytes depend on the support of fibroblasts in order to initiate colony formation" (page 421, left column, lines 18-20), and has no disclosure that motivates persons skilled in the art to separate the fibroblasts from the culture vessel before inoculating and culturing epithelial cells in the culture vessel. Therefore, even if a person skilled in the art referred to the cited references, he or she could not anticipate the present invention of Proposed Amended Claim 1.

Further, Applicants have prepared and attached a Declaration under 37 C.F.R. §1.132 to indicate the effect of the present invention. In Experiment 1, representing the present claimed invention, 3T3 mouse embryo fibroblasts are inoculated, incubated, then frozen to "kill" the fibroblasts. The killed fibroblasts are separated and then epidermal cells were inoculated into the culture vessel and incubated. In Comparative Experiment 1, 3T3 cells immortalized with mitomycin C are inoculated and then epidermal cells are inoculated into the culture flask with the 3T3 cells attached. This is also done in Comparative Experiment 2, but at a different starting density of 3T3 cells.

As shown in Figure 4 in the declaration, epidermal cells proliferate more in the present invention (i.e. Experiment 1) than in Comparative Experiments (about 1.55×10^6 cells in Experiment 1; about 9.0×10^5 cells and about 7.55×10^5 cells in Comparative Experiment 1 and 2, respectively). Also, it is shown in Figure 3 in the declaration that epidermal cells completely covered the culture surface in Experiment 1, but not in Comparative Experiments 1 and 2, 13 days after inoculation of

epidermal cells.

In the method of the cited references as well as Comparative Experiments 1 and 2 of the Declaration, epithelial cells are cultured in the presence of 3T3 mouse embryo fibroblasts. The Comparative Experiments are therefore representative of the cited references for purposes of comparison to the present invention. It is therefore understood that epithelial cells proliferate more in the present invention than in the cited references, and that the plating efficiency in the present invention is higher than in the cited references. Applicants submit that one of skill in the art would not have expected this result from the cited references of Boyce et al. or Rheinwald et al.

As mentioned above, it is understood from the descriptions in the cited references that fibroblasts are indispensable to initiate colony formation of keratinocytes. In addition, from the description in Boyce "only a range of two to six serial transfers were possible" (column 2, lines 38-39), it is understood that fibroblasts are preferable to be present in culture vessel during culturing keratinocytes. However, considering the descriptions in Boyce "low plating efficiency in primary culture (0.1 to 1.0%)" and "on subculture, the plating efficiency rose only occasionally to 10%" (column 2, lines 35-38), it is understood that the plating efficiency in Boyce's method in which fibroblasts are present in culture vessel is lower than that in the present invention. Also, based on low plating efficiency of Boyce's method, it is understood that epithelial cells proliferate less in Boyce's method than in the present invention. Therefore, based on the cited references, one of skill in the art would not expect the significant effect of the present invention.

Applicants therefore submit that the pending claims, as amended, are novel and non-obvious over Boyce et al. '666 or Rheinwald et al. (1977).

If, for any reason, it is felt that this application is not now in condition for allowance, the Examiner is requested to contact Applicants undersigned agent at the telephone number indicated below to arrange for an interview to expedite the disposition of this case.

In the event that this paper is not timely filed, Applicants respectfully petition for an appropriate extension of time. Please charge any fees for such an extension of time and any other fees which may be due with respect to this paper, to Deposit Account No. 01-2340.

Respectfully submitted,

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Enclosures: Declaration under 37 C.F.R. 1.132

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